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Fibrin monomer and fibrinopeptide B act additively to increase DNA synthesis in smooth muscle cells cultured from human saphenous vein

Justin Sturge, PhD, Nessa Carey, PhD, Alun H. Davies, DM, and **Janet T. Powell, MD,** *London, United Kingdom*

Purpose: **We investigated the hypothesis that fibrinogen increased DNA synthesis (and cell proliferation) of smooth muscle cells (SMCs) cultured from human saphenous vein and that the increased DNA synthesis was attenuated when cells were cultured on polymeric collagen.**
Methods: **SMCs were cultured from human saphenous vein on plastic, fibronectin, monomeric, and polymeric collagen. Fibrinogen products were prepared by proteolytic digestion. DNA synthesis was measured by bromodeoxyuridine incorporation into DNA, cell proliferation by cell counting, cyclic adenosine monophosphate by enzyme-linked immunosorbent assay, and fibrinopeptide B labeled with iodine 125 used for binding studies.**
Results: **Fibrin monomer (0.003-0.1 μmol/L) stimulated a concentration-dependent increase in DNA synthesis of up to 10-fold, which could be inhibited by the peptide Bβ15-42. The stimulation of DNA synthesis was highest for cells cultured on plastic and lowest for cells cultured on type I collagen polymer. Much higher concentrations of fibrinogen (0.3-1 μmol/L) were required to effect similar increases in DNA synthesis. Fibrinogen had a particular effect to augment DNA synthesis, up to 14-fold, when cells were cultured on monomeric type I collagen. This augmented DNA synthesis was inhibited by a neutralizing antibody to urokinase-type plasminogen activator. Incubation of cells cultured on collagen monomer with fibrinogen resulted in production of fibrinopeptide B. Fibrinopeptide B (5 μmol/L) increased DNA synthesis by fourfold and had additive effects with fibrin monomer to increase DNA synthesis. Iodinated tyrosine fibrinopeptide B bound to SMCs (dissociation constant 0.6 μmol/L).**
Conclusion: **Cultured human saphenous vein SMCs appear to have high-affinity receptors for fibrin monomer and fibrinopeptide B, the engagement of which stimulates DNA synthesis. These mechanisms may be pertinent to the association between fibrinogen and vein graft stenosis in vivo. (J Vasc Surg 2001;33:847-53.)**

Fibrinogen is a complex plasma protein with a molecular mass of approximately 350 kd that plays a central role in hemostasis and wound healing.[1] Several studies have indicated that elevated plasma levels of fibrinogen are associated with an increased risk of restenosis after angioplasty[2-4] and intimal lesion development and occlusion in saphenous vein bypass grafts.[5,6] These vascular pathologic conditions are characterized by rapid smooth muscle cell (SMC) proliferation and extracellular matrix remodeling. Fibrinogen can interact directly with SMC in the vessel wall, because it is deposited within the subendothelium and media of blood vessels and subjected to a physical insult, a perturbed shear flow, or both.[7-9]

Fibrinogen and its various cleavage products can stimulate differential mitogenic responses in fibroblasts, endothelial cells, and lymphoid cell lines, representing multiple fibrinogen ligand-receptor interactions that may be cell-type specific.[10-15] For example, exposure of the 15-42 sequence of the Bβ chains of fibrinogen (after release of fibrinopeptide B) is necessary for the mitogenic stimulation of anchorage dependent cells, such as endothelial cells and fibroblasts.[12] However, a similar requirement for fibrinopeptide B cleavage is not apparent in Raji cells, which are not anchorage dependent and proliferate in response to the intact fibrinogen molecule.[15] These differences may be attributed to the interaction of anchorage-dependent cells, through integrins, with their underlying matrix.

The gross changes in extracellular matrix composition during intimal lesion development disrupt normal SMC integrin-matrix interactions, alter intracellular signaling, and modulate the response of cells to growth promoting factors.[16-18] The ability of polymerized (fibrillar) type 1 collagen to inhibit DNA synthesis and cell proliferation[19,20] has been identified as an important regulatory factor that maintains a low proliferative index of SMC in the normal vessel wall. Indeed, inhibition of S-phase entry by polymeric type I collagen in human aortic SMCs uses an integrin-dependent signaling mechanism that is redundant in cells grown on monomeric (or soluble) type I collagen.[21] The mechanism of SMC proliferation also appears to depend on the activation of protein kinase A/cyclic adenosine monophosphate (cAMP) dependent signaling,[22] but it is not known whether this signaling is influenced by cell-matrix interactions.

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Reprint requests: Prof J. T. Powell, University Hospitals of Coventry & Warwickshire, Clifford Bridge Road, Coventry CV2 2DX (e-mail: Janet.Powell@wh-tr.wmids.nhs.wk).
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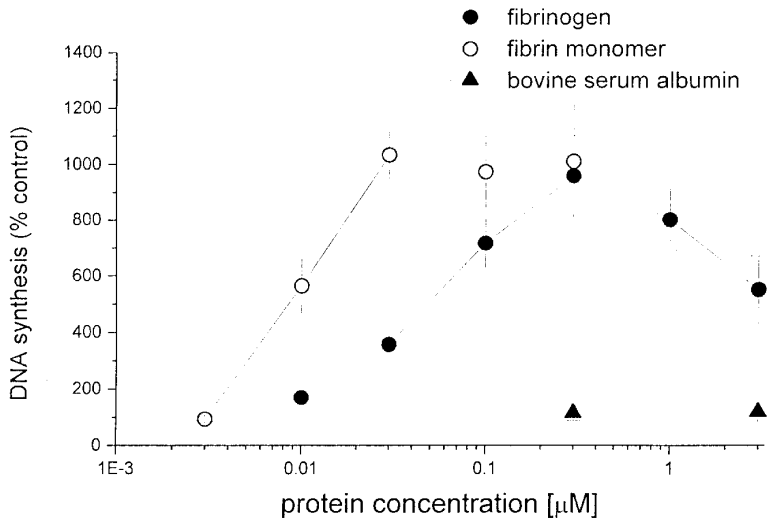

Fig 1. Fibrinogen and fibrin stimulation of DNA synthesis in SMCs cultured from human saphenous vein. Cells were cultured on uncoated plastic, and protein was added for 24 hours before measurement of DNA synthesis (BrdU incorporation). Results (mean ± SD) are reported as percentage DNA synthesis in comparison with controls with no added protein.

Table I. Effect of different proteins on cell proliferation

Cell number (× 10^{-3}) after 72 h					
Basal	3 μmol/L BSA	0.3 μmol/L fibrinogen	0.3 μmol/L fibrin monomer	0.1 μmol/L PDGF + 0.3 μmol/L BSA	1 U/mL thrombin + 0.3 μmol/L BSA
32 ± 4	39 ± 6	58 ± 11*	67 ± 19*	70 ± 13*	46 ± 7*

Cells (25×10^3) were cultured on uncoated plastic; results show mean ± SD of six replicate wells.
*Significant increase compared either with cells cultured in serum-free medium alone (basal) or in the presence of 3 mmol/L BSA, ANOVA $P < .03$.
ANOVA, Analysis of variance; *BSA,* bovine serum albumin; *PDGF,* platelet-derived growth factor.

Here we demonstrate that fibrinogen and some of its specific degradation products act as potent stimulators of DNA synthesis in cultured human vascular SMCs. We also indicate the potential role of the extracellular matrix in regulating the cellular response to fibrinogen.

MATERIALS AND METHODS

Cell culture. Saphenous vein was obtained from patients undergoing cardiovascular surgery at Imperial College, London, United Kingdom. For the studies described, veins were obtained from 18 separate donors, undergoing either coronary artery bypass (13) or femorodistal bypass (5) grafting, with approval of the Riverside Research Ethics Committee. Human saphenous vein SMC cultures were established by a modified version of the standard explant method.[23] Cells were cultured on either uncoated plastic or plastic coated with fibronectin, monomeric collagen, or polymeric collagen. Full details of the culture methods, preparation of fibrinogen and its cleavage products, measurement of DNA synthesis, cAMP, binding assays, and sources of reagents and peptides are given in the Appendix. DNA synthesis was measured by bromodeoxyuridine (BrdU) incorporation, cAMP was measured by immunoassay, and fibrinopeptides were quantified by capillary zone electrophoresis and radiolabeled with iodine 125.

Data analysis. The Student paired and unpaired *t* tests were used for comparison between experimental data points in all experiments.

RESULTS

Fibrinogen and soluble fibrin monomer stimulation of DNA synthesis in human saphenous vein SMCs. When cells were cultured on uncoated plastic, both fibrinogen and fibrin monomer stimulated DNA synthesis to a maximum of about 10-fold, although the concentration of fibrin monomer (0.03 μmol/L) required for peak DNA synthesis was less than the concentration of fibrinogen (Fig 1). Similar increases in DNA synthesis were observed when three commercial separate sources of fibrinogen, including one designated as thrombin free, were used. In contrast, the complex of the fibrinogen degradation products fragment D and fragment E had only a weak effect on DNA synthesis (data not shown), and bovine serum albumin (up to 4 μmol/L) had no effect (Fig 1). At concentrations lower than 1.0 μmol/L, fragment D and fragment E complex did

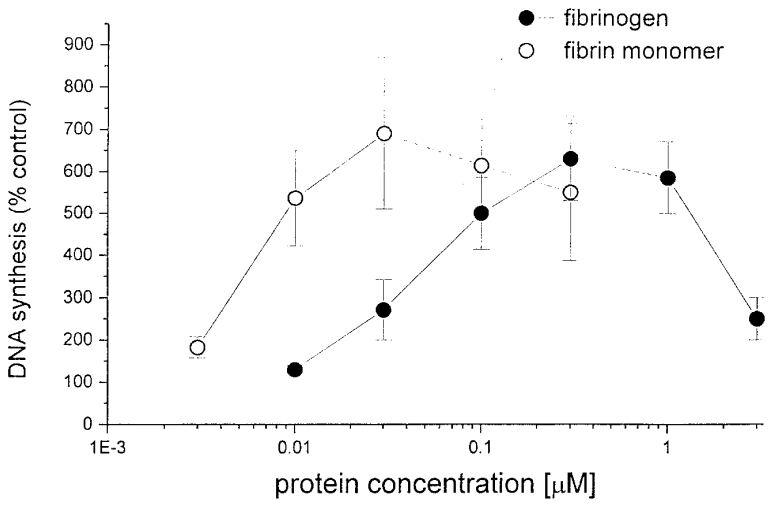

Fig 2. Stimulation of DNA synthesis in SMCs by fibrinogen and fibrin monomer for cells on fibronectin. Cells were cultured on fibronectin-coated plastic, and protein was added for 24 hours before measurement of DNA synthesis (BrdU incorporation). Results (mean ± SD) are reported as percentage DNA synthesis in comparison with controls with no added protein. Results for cells cultured on type I collagen polymer were almost identical, with superimposed curves.

not stimulate DNA synthesis. At 1.0 μmol/L fragment D and fragment E complex only stimulated a fourfold increase and at 3.0 μmol/L only stimulated a twofold increase in DNA synthesis (data not shown). Similarly, incubation of cells for 72 hours with either fibrinogen or fibrin monomer increased cell proliferation, in comparison to bovine serum albumin (Table I). Fibrin monomer was produced with thrombin (final concentration < 0.05 U/mL). This concentration of thrombin had no effect on DNA synthesis in cultured SMCs: even when used at 1 U/mL thrombin only stimulated a maximum twofold increase in DNA synthesis (data not shown) and a modest increase in cell number (Table I). Preincubation of cells with thrombin inhibitors, either hirudin (2 U/mL) or PPACK (phenylalanyl-L-prolyl-L-arginyl-chlormomethylketone) (1 μmol/L) did not alter the concentration-dependent increase in DNA synthesis in response to fibrinogen. The fibrin polymerization inhibitor GPRP (glycine-proline-arginine-proline) (0.01-3.0 mmol/L) did not alter DNA synthesis stimulated by fibrin monomer. Therefore, it appeared likely that both fibrin monomer and fibrinogen could stimulate DNA synthesis in SMCs cultured from human saphenous vein. Incubation of cells with 0.3 μmol/L fibrinogen resulted in a significant decrease in cellular cAMP concentration, to 0.94 ± 0.21 pmol per million cells compared with 1.60 ± 0.15 in the absence of fibrinogen (n = 4, $P < .05$). In contrast, incubation of cells with fibrin monomer (0.03 μmol/L) had no effect on cAMP concentration.

Fibrinogen stimulation of DNA synthesis on collagen monomer, collagen polymer, and fibronectin. Concurring with previous studies,[19-21] culture of SMCs on type I collagen polymer inhibited serum-stimulated DNA synthesis by about 50% compared with culture on uncoated plastic. Therefore, DNA synthesis was adjusted to the percentage basal levels measured on each particular matrix. The concentration-dependent effects of fibrinogen on increased DNA synthesis were reduced when SMCs were cultured on plastic coated with either collagen polymer or fibronectin, with 0.3 μmol/L fibrinogen stimulating only a fourfold to sixfold increase in DNA synthesis. Again, inclusion of either PPACK (1 μmol/L) or hirudin (2 U/mL) in the culture medium did not alter the response. Similarly, fibrin monomer only stimulated a maximum fourfold to sixfold increase in DNA synthesis when SMCs were cultured on either collagen polymer or fibronectin. However, the shape of the concentration-response curves was similar to that for SMCs cultured on plastic (the data for cells cultured on fibronectin are shown in Fig 2). In contrast, when SMCs were cultured on collagen monomer, the response to fibrinogen, but not to fibrin monomer, was altered markedly (Fig 3). However, the inclusion of PPACK in the culture media returned the concentration-response curve to one similar to the curves on fibronectin (Figs 2 and 3). PPACK did not alter fibrinogen-mediated increases in DNA synthesis when SMCs were cultured on plastic, fibronectin, or collagen polymer. Parallel to the results for SMC cultured on uncoated plastic, fibrinogen, but not fibrin monomer, caused a significant 35% decrease in the concentration of cAMP of cells cultured on collagen monomer. The basal concentration of cAMP, 1.55 ± 0.2 pmol per million cells, was unchanged 30 minutes after the addition of 0.1 μmol/L fibrin monomer but decreased to 1.05 ± 0.1 pmol per million cells after addition of 0.1 μmol/L fibrinogen ($P < .05$, n = 4).

The role of fibrinopeptide cleavage for cells cultured on collagen monomer. To investigate whether the enhanced response to fibrinogen for SMCs cultured on collagen monomer depended on a cell-associated serine

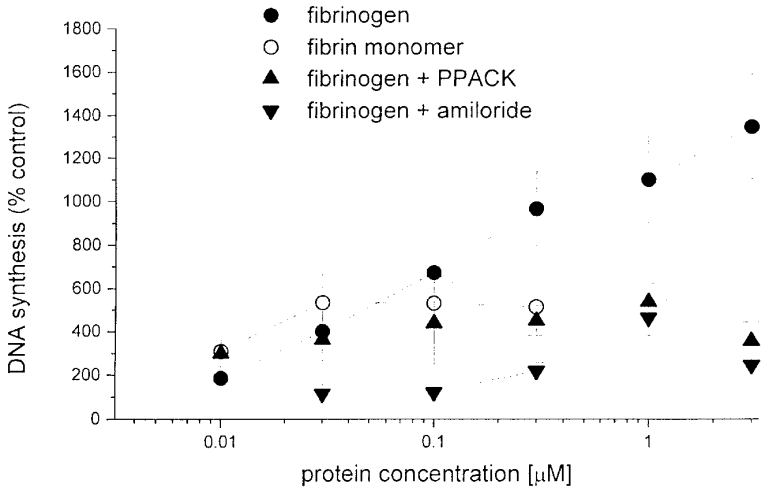

Fig 3. Inhibition of the fibrinogen-mediated stimulation of DNA synthesis for SMCs cultured on monomeric collagen. Cells have been cultured on plastic coated with collagen monomer, and protein was added for 24 hours before measurement of DNA synthesis (BrdU uptake). Amiloride was used at 10 μmol/L and PPACK at 1 μmol/L. Results (mean ± SD) are reported as percentage DNA synthesis in comparison with controls with no added protein.

Table II. Effect of u-PA inhibition on fibrinogen-stimulated DNA synthesis in SMCs cultured on type collagen monomer and polymer

Substratum	*Monomeric type I collagen*	*Polymeric type I collagen*
Basal (no fibrinogen)	100	100
Fibrinogen (0.3 μmol/L)	710 ± 117	390 ± 120
+Amiloride	275 ± 21	290 ± 95
+Benzamidine	460 ± 158	395 ± 115
+Anti–u-PA	485 ± 62	415 ± 75
+Anti–MCP-1	805 ± 130	460 ± 67

DNA synthesis is reported as percentage of basal levels in the absence of added fibrinogen. Results (mean ± SD) are from four to eight separate experiments.

protease, we included hirudin (a thrombin inhibitor) amiloride (which inhibits urokinase-type plasminogen activator [u-PA]) and benzamidine (a general serine protease inhibitor) in the SMC culture medium. None of these compounds altered DNA synthesis in the absence of fibrinogen. The presence of amiloride (10 μmol/L) significantly inhibited DNA synthesis stimulated by fibrinogen (0.03-0.3 μmol/L) in cells cultured on type I collagen monomer, but had no effect on DNA synthesis stimulated by similar concentrations of fibrinogen in cells cultured on type I collagen polymer (0.3 μmol/L fibrinogen, Table II). Benzamidine (1 mmol/L) also significantly inhibited DNA synthesis stimulated by fibrinogen (0.1-0.3 μmol/L) in cells cultured on type I collagen monomer but had no effect on DNA synthesis stimulated by similar concentrations of fibrinogen in cells cultured on type I collagen polymer (0.3 μmol/L fibrinogen, Table I). Hirudin did not alter the fibrinogen-mediated stimulation of DNA synthesis. The significant effect of amiloride suggested the involvement of u-PA. A neutralizing antibody to u-PA (10 μg mL^{-1}) significantly inhibited DNA synthesis stimulated by fibrinogen (0.1-0.3 μmol/L) in cells cultured on monomeric but not polymeric type I collagen (Table II), whereas an isotype-matched antibody (anti–monocyte chemoattractant protein-1 [anti-MCP-1]) had no effect. Taken together, these results indicate that u-PA is actively involved in the additional DNA synthesis stimulated by fibrinogen in cells cultured on type I collagen monomer.

Because u-PA has been shown to release fibrinopeptide B from fibrinogen in vitro,[24] we measured fibrinopeptide B accumulation in the conditioned culture medium of cells incubated with fibrinogen, using capillary zone electrophoresis. Fibrinopeptide B generation was significantly higher in cells grown on type I collagen monomer compared with plastic (0.77 ± 0.06 μmol/L and 0.43 ± 0.08 μmol/L, respectively at 3 μmol/L fibrinogen, $P < .05$). Fibrinopeptide A remained below the limits of detection. The presence of amiloride (10 μmol/L) inhibited fibrinopeptide B accumulation in the conditioned medium of cells grown on type I collagen monomer to levels below the detection limits of the assay in the presence of 1 μmol/L fibrinogen and to 0.16 ± 0.09 μmol/L in the presence of 3 μmol/L fibrinogen. These findings imply that when human saphenous vein SMCs are cultured on collagen monomer there is enhanced u-PA activity, which permits the cleavage of fibrinopeptide B from fibrinogen.

Fibrinopeptide B and stimulation of DNA synthesis in human saphenous vein SMCs. Fibrinopeptide B (1-10 μmol/L) stimulated DNA synthesis in SMCs to a maximum of fivefold, and results were similar for cells cultured on uncoated plastic, fibronectin collagen polymer, or monomer (data not shown). The tyrosine deriva-

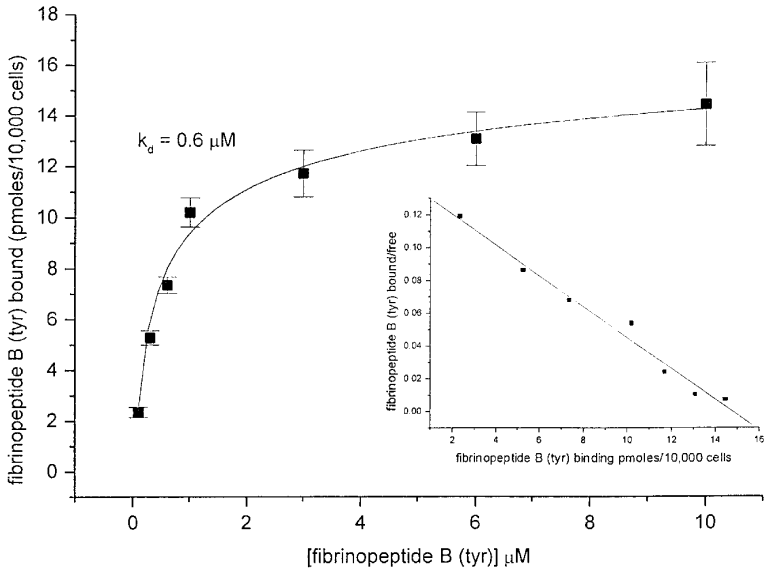

Fig 4. The binding of labeled fibrinopeptide B to SMCs cultured from human saphenous vein. Iodinated fibrinopeptide B-tyr was incubated at 4°C with confluent monolayers of SMCs cultured on uncoated plastic. Results show saturable binding (mean ± SD for 5-6 wells). Scatchard plot (*inset*) suggests presence of a single, high-affinity, binding site, with an apparent K_d of 0.6 μmol/L calculated from binding curve. *B (tyr)*, Tyrosine derivative of fibrinopeptide B.

Table III. The effects of fibrin monomer and fibrinopeptide B on DNA synthesis in SMCs cultured on different substrata

	DNA synthesis (% control)			
Substratum	*Plastic*	*Fibronectin*	*Collagen monomer*	*Collagen polymer*
Fibrinopeptide B (5 μmol/L)	387 ± 108	412 ± 146	485 ± 96	306 ± 155
Fibrinogen (3 μmol/L)	759 ± 178	411 ± 92	515 ± 93	433 ± 46
Fibrinogen (3 μmol/L) + FPB	746 ± 95	435 ± 76	508 ± 78	448 ± 80
Fibrin monomer (0.3 μmol/L)	834 ± 213	447 ± 92	535 ± 64	393 ± 141
Fibrin monomer (0.3 μmol/L) + FPB	1154 ± 188*	856 ± 105*	930 ± 233*	402 ± 126
Fibrin monomer (0.3 μmol/L) + FPA	636 ± 121	493 ± 202	358 ± 104	306 ± 155

Results (mean ± SD) are from 3-15 separate experiments. All experiments using fibrinogen were carried out in the presence of 1 μmol/L PPACK.
*Significantly higher than for fibrin monomer only, $P < .02$, paired t test.
FPB, Fibrinopeptide B at 5 μmol/L; *FPA*, fibrinopeptide A at 5 μmol/L; *SMC*, smooth muscle cell.

tive of fibrinopeptide B (fibrinopeptide B-tyr) had a similar concentration-dependent effect on DNA synthesis as fibrinopeptide B (data not shown). Fibrinopeptide B (5 μmol/L) did not appear to alter the cellular cAMP concentration. Fibrinopeptide B, but not fibrinopeptide A, and fibrin monomer showed additive effects to increase DNA synthesis in human saphenous vein SMCs cultured on type I collagen monomer (Table III). Separately, fibrin monomer (0.3 μmol/L) and fibrinopeptide B (5 μmol/L) stimulated fivefold and fourfold increases in DNA synthesis respectively; together a ninefold increase was observed (Table III). In contrast, fibrinopeptide B and fibrinogen (0.01-1.0 μmol/L), in the presence of PPACK, did not have additive effects (Table III). Similar additive effects of fibrinopeptide B and fibrin monomer were observed for cells cultured on plastic and fibronectin, but not for cells cultured on type I collagen polymer. These experiments strongly suggest that human saphenous vein SMCs have specific receptors for both fibrin monomer and fibrinopeptide B, the engagement of which stimulates DNA synthesis. Experiments with [^{125}I]fibrinopeptide B-tyr were performed to clarify whether fibrinopeptide B bound to SMCs. Like fibrinopeptide B, fibrinopeptide B-tyr stimulated DNA synthesis in SMCs. Saturable binding of [^{125}I]fibrinopeptide B-tyr peptide to confluent monolayers of SMCs cultured on plastic was demonstrated, an apparent dissociation constant (K_d) of 0.6 μmol/L at 4°C (Fig 4). The binding of fibrinopeptide B-tyr was reversed by a 100-fold molar excess of fibrinopeptide B. Competition experiments with fibrinogen were restricted by the limited solubility of this protein. Scatchard plot analysis (Fig 4, *inset*) indicates the presence of a single-

Table IV. Summary results

Fibrinogen/derivative	*Stimulation of DNA synthesis*	*Cellular cAMP*
Fibrinogen	↑↑ (P, CP, F) ↑↑↑↑ (CM)	↑
Fragment D, fragment E	↑ (P, CM)	ND
Fibrin monomer	↑↑ (P, CM, CP, AN)	↔
Fibrinopeptide B	↑↑ (P, CM)	↔

cAMP, cyclic adenosine monophosphate; *CM,* Collagen monomer; *CP,* collagen polymer; *F,* fibronectin; *ND,* not determined; *P,* uncoated plastic.

binding site for fibrinopeptide B-tyr on SMCs cultured from human saphenous vein. The binding experiment shown in Fig 4 was repeated once, with similar results (K_d 0.65 μmol/L).

Inhibition of fibrin-mediated increases in DNA synthesis. In contrast to fibrinopeptide B, the peptides RGDV, γ3, Bβ15-42, or a scrambled isomer of the peptide Bβ15-42 at low concentrations (10 μmol/L) had no effect on DNA synthesis of SMCs. At high concentrations (50 μmol/L), Bβ15-42 and its scrambled isomer increased DNA synthesis by up to 50%. Only Bβ15-42 (50 μmol/L) significantly inhibited (by 30%-40%) the effect of fibrin monomer (0.03-0.1 μmol/L) to increase DNA synthesis and cell proliferation (data given in Appendix, online only). This peptide (50 μmol/L) had a negligible effect on fibrinogen-stimulated DNA synthesis.

The effect of fibrinogen and its products on DNA synthesis in saphenous vein SMCs. A summary of the effects of all the different fibrinogen products on DNA synthesis and cellular cAMP concentration is given in Table IV.

DISCUSSION

This is the first study to report that fibrin monomer has potent effects on DNA synthesis and cell proliferation in human vascular SMCs, although previous studies have shown similar effects in rabbit SMCs.[25] Moreover, fibrinopeptide B and fibrin monomer have additive effects to increase, by an order of magnitude, DNA synthesis in SMCs cultured from human saphenous vein. Fibrinogen also increased DNA synthesis, but much higher concentrations were required than of fibrin monomer. These findings suggest a possible mechanism underlying the association of increased plasma concentrations of fibrinogen with restenosis after angioplasty and vein graft stenosis, with intimal SMC proliferation being an essential feature of both pathologic conditions. The generation of fibrin monomer and fibrinopeptide B close to the surface of SMCs may arise through either thrombin activity or the activity of enzymes such as u-PA, at the cell surface. We also provide evidence that, in cultured SMCs, cell-derived u-PA degrades fibrinogen and generates products with enhanced effects on the stimulation of DNA synthesis. The modulation of these processes by the extracellular matrix is in keeping with the situation in intimal hyperplasia, where SMCs of a synthetic phenotype proliferate in an amorphous matrix that is likely to include soluble collagen and collagen degradation products.

It is unlikely that our findings are confounded by the possibility that fibrinogen preparations were contaminated with mitogens such as thrombin or basic fibroblast growth factor.[26] First, thrombin, even at concentrations of 1 U/mL, only stimulated DNA synthesis in SMCs cultured from saphenous vein by a maximum of twofold. Second, the binding of basic fibroblast growth factor to fibrinogen and fibrin monomer is similar,[26] but we observed that fibrin monomer was 10- to 100-fold more potent as a mitogen for SMCs compared with fibrinogen (Figs 1 and 2). Moreover, the effects of fibrin monomer could be selectively inhibited by the peptide derived from the newly exposed aminoterminus of the Bβ fibrinogen chains, Bβ15-42. This latter observation also indicates the presence of specific receptors for fibrin monomer on the SMC plasma membrane. The possible involvement of the Bβ15-42 sequence of fibrin monomer is strengthened by two further observations. First, the fibrinogen fragment complex fragment D and fragment E, which lacks the Bβ15-42 sequence, had no effect to increase DNA synthesis. Second, a scrambled isomer of the peptide Bβ15-42 did not inhibit fibrin-mediated increases in DNA synthesis. The SMC receptor for the Bβ15-42 epitope of fibrin monomer will be investigated in future studies.

The remarkable increase in DNA synthesis stimulated by fibrinogen for cells cultured on collagen monomer revealed a possible mechanism relevant to the proliferation of vascular SMCs in intimal lesions in vivo. The altered extracellular matrix associated with vascular injury (increase in soluble type I collagen and decrease in fibrillar type I collagen) is known to be important in the regulation of vascular SMC growth in response to various mitogenic stimuli.[21] In vitro it has been shown that the enzyme u-PA can directly cleave fibrinopeptide B, but not fibrinopeptide A, from fibrinogen.[24] Inhibitors of u-PA (amiloride, benzamidine, and anti–u-PA antibodies) inhibited fibrinogen-stimulated DNA synthesis by 35% to 60% in cells cultured on type I collagen monomer but on no other substrata and prevented the accumulation of fibrinopeptide B. These findings indicate that the activity of SMC u-PA is increased when cells are cultured on collagen monomer.

We did not use scrambled peptides of fibrinopeptide B to determine whether its mitogenic effects on SMCs were specific. However, we have determined that fibrinopeptide B has a single-receptor binding site on human saphenous vein SMCs with a binding affinity (K_d = 0.6 μmol/L), similar to the concentration of fibrinopeptide B released from fibrinogen by cells cultured on collagen monomer. Fibrinopeptide B has been reported previously to have mitogenic properties, but without firm evidence for the presence of a cellular receptor for this fibrinogen degradation product.[11,27,28] Moreover, fibrinopeptide B (but not fibrinopeptide A) and fibrin monomer had additive effects on DNA synthesis for cells cultured on fibronectin, collagen monomer, or uncoated plastic.

In summary, we have shown that fibrin monomer is a potent mitogen for vascular SMCs cultured from human saphenous vein. These cells also appear to have receptors for fibrinopeptide B, the engagement of which provides for the additive effects of this peptide and fibrin monomer to augment DNA synthesis. We have described these effects using cultured cells, but similar mechanisms could be important to the development of intimal hyperplasia in vivo.

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